(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 February 2003 (27.02.2003)

PCT

(10) International Publication Number
WO 03/015923 A1

(51) International Patent Classification[7]: B01L 3/02, 3/00, F04B 43/10, 45/00, 43/06, B01F 13/02, 11/00, 13/02, 7/00, 5/06

(21) International Application Number: PCT/US02/25743

(22) International Filing Date: 13 August 2002 (13.08.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/313,703	20 August 2001 (20.08.2001)	US
60/339,851	12 December 2001 (12.12.2001)	US
PCT/US02/07113	8 March 2002 (08.03.2002)	US
10/211,503	2 August 2002 (02.08.2002)	US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US PCT/US02/07113 (CIP)
Filed on 8 March 2002 (08.03.2002)

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,

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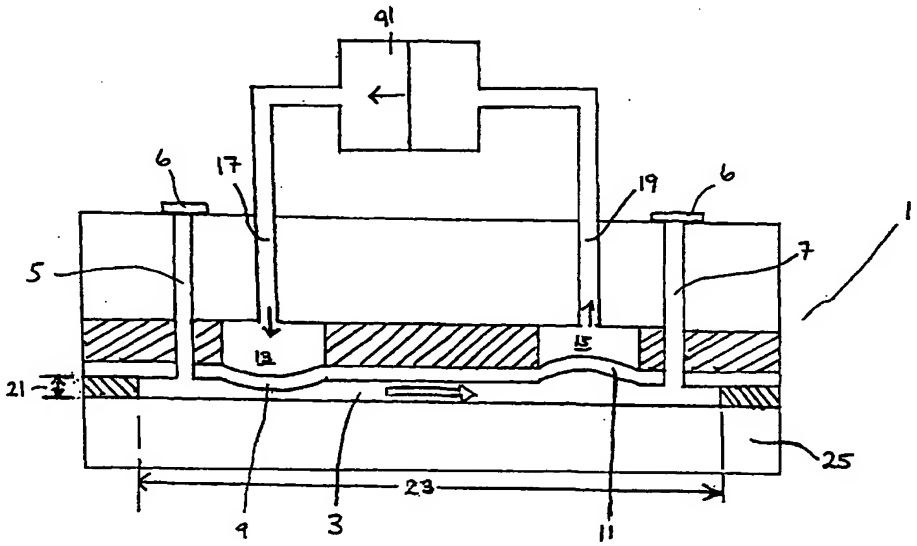
(54) Title: FLUID MIXING IN LOW ASPECT RATIO CHAMBERS



(57) Abstract: A method and system for performing mixing in a low volume, low aspect ratio microfluidic chamber (3) is described. Two or more mixing bladders (13,15) formed adjacent the microfluidic chamber are inflated and deflated in reciprocating fashion to cause inward and outward deflection of discrete regions of the chamber wall to mix fluid within the chamber. Mixing bladders are actuated by air or another gas, or by a liquid such as water, pumped in and out of the bladders with a pump which may be located remote from the microfluidic device including the microfluidic chamber. In an alternative embodiment, mixing is generated by applying alternating mechanical forces to a surface of a flexible chamber forming device. The microfluidic chamber may be a hybridization chamber formed on a microarray (25) slide with the use of a microarray interface device, or it may be a microfluidic chamber formed in various other types of microfluidic devices.

GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

FLUID MIXING IN LOW ASPECT RATIO CHAMBERS

Related Applications

This application is a continuation-in-part of PCT Application PCT/US02/07113, filed March 8, 2002, which application claims the benefit of U.S. Provisional Application 60/274,389, filed March 9, 2001, U.S. Provisional Application 60/284,427, filed April 17, 2001, U.S. Provisional Application 60/313,703, filed August 20, 2001, and U.S. Provisional Application 60/339,851, filed December 12, 2001. It is also a continuation-in-part of PCT Application PCT/US02/_____, filed August 2, 2002 and identified by Attorney Docket No. 3153.2.17. The foregoing applications are incorporated by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to the area of microfluidic devices. In particular, it relates to methods and systems for agitating or mixing fluids in chambers of microfluidic devices. More particularly, it relates to methods and systems for generating agitation or mixing in low aspect ratio chambers formed on substrates bearing immobilized biological or biochemical samples or compounds.

Description of Related Art

A variety of biological and chemical assays have been developed for detecting the presence of compounds of interest in samples. In the biomedical field, methods for detecting the presence of specific nucleotide sequences, proteins or peptides are utilized, for example, in diagnosing various medical conditions, determining predisposition of patients to diseases, and performing DNA fingerprinting. In general, biological and chemical assays are based on exposing an unknown sample to one or more known reactants and monitoring the progress or measuring the outcome of the reaction. There is currently a high level of interest in the development of high throughput methods for performing multiple biological and chemical analyses of this type simultaneously, quickly, and conveniently.

One recently developed method for performing multiple chemical reactions simultaneously is to form a microarray of multiple spots of reactant molecules on a planar substrate such as a glass microscope slide, typically in a two-dimensional grid pattern, and apply liquid reagents and reactants to the slide to contact multiple spots simultaneously. Various reaction steps may be performed with the bound molecules in the microarray, including exposure of bound reactant molecules to liquid reagents or reactants, washing,

and incubation steps. It is typical to immobilize known reactants on the substrate, expose an unknown liquid sample to the immobilized reactants, and query the reaction products in order to characterize the sample. However, it is also possible to immobilize one or more unknown samples on the substrate and expose them to a liquid containing one or more known reactants.

Microarrays are frequently used in analysis of DNA samples, but may also be used in diagnostic testing of other types of samples. Spots in microarrays may be formed of various large biomolecules, such as DNA, RNA, and proteins, smaller molecules such as drugs, co-factors, signaling molecules, peptides or oligonucleotides. Cultured cells may also be grown onto microarrays. As an example, if it is desired to detect the presence of particular DNA sequences in a patient sample, the sample is exposed to a microarray of spots formed of oligonucleotides having sequences complementary to sequences of interest. The occurrence of hybridization between the sample and a known sequence in a particular spot then indicates the presence and perhaps, additionally the quantity, of the sequence associated with that spot in the sample.

Microarrays offer great potential for performing complex analyses of samples by carrying out multiple detection reactions simultaneously. However, some of the current limitations of microarrays are the time and care required to process slides, the difficulty in obtaining consistent, high quality results, and limited sensitivity, which makes detection of low-expression genes difficult. The need for high quality microarray processing is particularly pronounced because individual microarray slides are expensive and only limited quantities of the samples used in the reactions may be available, making it particularly important to obtain good results consistently.

It is often desirable that reactions performed on microarrays consume minimal quantities of sample, due to the limited sample availability, as noted above. However, when small quantities of sample fluid are spread out over the area of the microarray, the fluid layer is very thin, leading to the possibility that, if no mixing is provided, the sample fluid will become locally depleted of a particular sequence over the spot binding that sequence. As target is depleted, reaction kinetics slow, resulting in a lower signal. This is a greater problem for low-abundance sequences. It is considered particularly desirable that hybridization be performed in a low-volume chamber, with mixing. Low volumes allow for higher concentration of reactants that are in limited supply, while mixing maintains initial kinetic rate and thus produces more reaction products.

A number of approaches have been proposed for providing mixing on microarray slides. These include applying acoustic energy to the hybridization mixing (e.g., PCT publication No. WO/0170381 or U.S. Patent No. 5,922,591), or pumping fluids in and out of the hybridization chamber or pumping fluids back and forth between several chambers or between separate compartments of a single chamber (e.g., PCT publication WO/0201184, U.S. Patent No. 5,922,591), sometimes with the inclusion of dividers, particles, or bubbles within the chamber to enhance mixing. However, these methods depend on the use of relatively large sample volumes, large hybridization chambers, and inconvenient or complicated equipment, and in some cases require the use of specially designed slides or other substrates. There remains a need for a system that provides a mixing function in a low volume hybridization chamber suitable for use with common microarray slides.

SUMMARY OF THE INVENTION

The present invention is a method and system for providing mixing in low-volume hybridization chambers suitable for use with common microarray slides. The mixing technology according to the invention may be incorporated into several types of microarray interface devices suitable for forming low-volume hybridization chambers, as described in commonly-owned patent applications PCT Application PCT/US02/07113, filed March 8, 2002 and PCT Application PCT/US02/_____, filed August 2, 2002.

The novel mixing mechanism is not limited to use in hybridization chamber devices, and may also be incorporated into other types of fluid handling devices to generate fluid movement and mixing in many types of microfluidic chambers or channels, particularly those having low aspect ratio chambers or channels having at least one microscale dimension.

Mixing according to the invention is produced by the inflation and deflation of mixing bladders located adjacent to the microfluidic chamber or channel causing inward or outward deflection of the chamber wall, and consequently displacement of fluid within the chamber. Inward deflection of the chamber wall in one region of the chamber is accompanied by outward deflection of the chamber wall in another region of the chamber, so that the total volume of the chamber remains substantially constant. The mixing bladders may be formed from recesses or openings in the device structure covered by a thin, flexible membrane. The flexible membrane may be formed integrally with the material in which the recess is formed, or may be a sheet of flexible sheet material adhered thereto.

Bladders are activated by positive and negative air (or other gas) pressures that may be generated remote to the microfluidic device. Alternatively, water or other liquid may be pumped in and out of the bladders to generate deflections of the chamber wall. Bladders and associated air (fluid) lines can be incorporated into the design of microfluidic devices relatively simply and inexpensively, and therefore are suitable for use in devices intended for one-time use.

In a further alternative embodiment of the device, externally applied mechanical forces may be used to deflect the chamber wall and thus produce mixing in low aspect ratio chamber.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a schematic cross-sectional diagram of a microfluidic chamber with adjacent mixing bladders.

FIG. 2 is an exploded view of a microarray slide and first embodiment of an interface device for forming a microfluidic chamber on the slide surface.

FIG. 3 is a transparent exploded view of a microarray slide and a second embodiment of an interface device for forming a microfluidic chamber on the slide surface.

FIG. 4 is a schematic cross-sectional view of a microarray slide and interface device as depicted in FIG. 3.

FIG. 5 is a top view of the interface device of FIG. 2 attached to a slide.

FIG. 6 illustrates the use of manifolds to connect positive and negative pressure sources to multiple interface devices.

FIG. 7 is a cross sectional view of the interface device of FIG. 2, showing clamping of the device to air lines from a pressure source.

FIG. 8 depicts an exemplary barbed fitting for connecting an air tube to an interface device.

FIG. 9 is a cross-sectional view of an embodiment of the device in which pressure is actively controlled in one mixing bladder.

FIG. 10 illustrates a microarray interface device having two narrow passive diaphragms.

FIG. 11 illustrates a device configuration in which both mixing bladders are alternately switched between a pressure source and the atmosphere.

FIG. 12 is a schematic cross-sectional view of another alternative embodiment of the invention.

FIG. 13 is an exploded view of a system in which two mixing bladders overlie multiple reaction chambers.

FIG. 14 depicts an alternative method of forming mixing bladders.

FIG. 15 illustrates the application of mechanical pressure to generate mixing.

FIGS. 16A and 16B illustrate an alternative method of applying mechanical pressure to generate mixing.

DETAILED DESCRIPTION OF THE INVENTION

The invention is illustrated in the schematic view of FIG. 1, which is a cross-sectional schematic of a microfluidic device 1 having a low aspect ratio chamber 3. Inlet port 5 and outlet port 7 provide for the introduction and removal, respectively, of fluids to and from chamber 3. Thin diaphragm regions 9 and 11 are located between chamber 3 and mixing bladders 13 and 15, respectively, and are displaced into and out of chamber 3 by inflation and deflation of mixing bladders 13 and 15 to generate fluid movement within chamber 3. An increase of pressure in mixing bladder 13 leads to deflection of diaphragm region 9 into chamber 3, while a simultaneous decrease of pressure in mixing bladder 15 leads to deflection of diaphragm region 11 out of chamber 3, to cause fluid movement in the direction indicated by the arrow. Similarly, an increase in pressure in mixing bladder 15 coupled with a decrease in pressure in mixing bladder 13 leads to inward deflection of diaphragm region 11 and outward deflection of diaphragm 9 with respect to chamber 3, to produce fluid movement in the opposite direction from the arrow. Each mixing bladder 13 and 15 has at least one air connection 17 or 19, respectively, which communicates with a pressure source 91 that provides for air (or other gas or liquid) to be moved in and out of the mixing bladders. Chamber 3 has a height 21 that is small with respect to its length 23. The ratio of chamber height 21 to chamber length 23 may range from about 1:30 to about 1:10,000, is preferably between about 1:100 and about 1:1,000, and is most preferably between about 1:200 and about 1:300. Height 21 of chamber 3 is also small with respect to the width of chamber 3. The height 21 of chamber 3 will have a microscale dimension ranging from a few microns to several hundred microns. Typical dimensions are in the range of about 10 to about 500 μm, although for different applications, the preferred range may vary, and devices according to the present invention may be constructed with chamber heights of as much as 1 or more millimeters. For hybridizations performed on standard microarray slides, a chamber height of about 10 μm to about 50 μm is preferred, with a height of between about 10 μm and about 37 μm being more preferred, and a height of

about 15 μm to about 30 μm being most preferred. For other applications, in which the chamber contains bulkier reactant materials, such as cells, tissue, beads, etc., a larger chamber height may be preferred, e.g., in the range of about 50 to about 1000 μm, and most preferably 50 to 300 μm. The preferred chamber height may also depend on the volume and concentration of available sample. The chamber width will typically be smaller than the chamber length. A number of different chamber configurations will be described in connection with particular embodiments of the invention.

Example 1

FIG. 2 depicts a first exemplary embodiment of the invention in the form of an adhesive laminate microarray interface device designed for performing hybridizations on a conventional 1" x 3" microarray slide. The device illustrated in FIG. 2 is described more fully in commonly owned patent application PCT/US02/_____ (Attorney Docket No. 3153.2.17). The microarray interface device 1 is designed to attach to a glass microarray slide 25 by means of an adhesive gasket 27 to form a hybridization chamber, equivalent to corresponding chamber 3 in FIG. 1, which contains a microarray of arrayed spots 26 of DNA or other material of interest. The device is useful for microarray processing, because providing mixing of labeled target solution during the hybridization process in a low volume hybridization chamber may lead to improved sensitivity and more reproducible microarray processing. The microarray interface device is particularly designed for use with conventional microarray slides, but may also be used to process cytology or histology slides, or slides with any other types of material that are processed by delivering fluids to the surface of the slide. The chamber on the slide surface is referred to as a hybridization chamber when the device is used with slides on which DNA hybridization is performed. When used with for other applications, the chamber may be described simply as a reaction or processing chamber.

The microarray interface device shown in FIG. 2 is formed of a top layer 29, an intermediate adhesive layer 31, a diaphragm layer 33, and an adhesive gasket 27. Adhesive gasket 27 defines the height, width, and length of the hybridization chamber. The length and width of the chamber are defined by opening 35 in gasket 27, which is preferably large enough to accommodate all of the arrayed spots 26. The ends of opening 35 may be tapered at ends 66 and 68, as shown, to prevent the entrapment of air during loading of liquid into the chamber. It is usually preferred that the thickness of adhesive gasket 27, which defines the height of chamber 3, is as small as possible to reduce the volume of the chamber. Exemplary dimensions for the chamber are a 25 μm high, 20 mm wide, and 60 mm long. A

chamber formed as depicted in FIG. 2 and having these dimensions will have a volume of about 38 µl. As noted previously, the height of the chamber (i.e., the smallest dimension, whether the chamber as a whole has a vertical, horizontal, or angled orientation) may range in size from a few microns to several hundred microns. A low chamber height means that a smaller sample volume will be required in order to cover a microarray having a particular length and width. However, a number of factors influence the lowest possible height for the chamber. If the chamber height is too small, it may be difficult to fill the chamber with sample. Also, smoothness of the slide surface and the chamber wall are critical when the chamber height is small, since uneven surfaces will cause non-uniform flow of fluid into the chamber, leading to trapping of air bubbles, and uneven thickness of the fluid layer on the microarray once the chamber has been filled. If the chamber height is too large relative to the size and flexibility of the diaphragms, the displacement of the diaphragms into the chamber will not displace a large enough percentage of the chamber volume to generate efficient mixing. The length and width of the chamber can be considerably larger than the height, with the length greater than or equal to the width. The length may range from about 1 cm to about 20 cm, and more typically from about 2 cm to about 7 cm, and the width may range from about 1mm to about 10cm, or more typically about 1 cm to about 2 cm. The volume of the reaction chamber of the inventive device may vary over several orders of magnitude, depending on the particular chamber dimensions selected, ranging from low values on the order of a microliter to maximum values on the order of a milliliter. Preferred volumes for hybridizations performed on conventional microarray 1"x 3" slides will range from about 10 to about 50 µl.

The device in FIG. 2 has gasket 27 that provides a perimeter wall about the low aspect ratio chamber, with surface 37 of diaphragm layer 33 forming a substantially planar first main wall of the chamber, and surface 39 of slide 25 forming a substantially planar second main wall of the chamber. Diaphragm layer 33 typically defines the upper surface of the hybridization chamber 3. As shown in FIG. 1 portions of diaphragm layer 33 function as flexible diaphragm regions 9 and 11, which form the lower surface of mixing bladders 13 and 15. Referring now to FIG. 2, the mixing bladders are defined by openings 45 and 47 in intermediate adhesive layer 31, and bounded below by diaphragm layer 33 and above by top layer 29. Air channels 49 and 51 are also defined by intermediate adhesive layer 31 and bounded below by diaphragm layer 33 and above by top layer 29. Holes 53 and 55 provide access to air channels 49 and 51, respectively, through diaphragm layer 33, for connection of mixing bladders to a pressure source. Possible pressure sources will be

described in detail subsequently. Holes 57 and 59 in top layer 29, holes 61 and 63 in intermediate adhesive layer 31, and holes 65 and 67 in diaphragm layer 33 are aligned to form inlet port 5 and outlet port 7, respectively, through which fluids enter and exit the hybridization chamber. Referring to FIG. 1, inlet port 5 and outlet port 7 are preferably small to minimize the dead volume contained within. Referring to FIG. 2, inlet port 5 and outlet port 7 preferably connect to opposite ends 66 and 68 of opening 35, respectively. Inlet port 5 and outlet port 7 are located close enough to the edge of opening 35 that they are supported by the adhesive gasket 27 so that they do not collapse against the slide surface 39 when pressure is applied by a pipette or micro syringe used to introduce solution into the hybridization chamber.

The device of FIG. 2 is formed of semi-rigid stock sheet and film materials that are made of neutral hydrophobic/hydrophilic materials, such as copolyester, Kapton™, polycarbonate, polystyrene, polypropylene, etc. Diaphragm layer 33 may be formed of polyester film, polyether imide, or polyimide. Adhesive gasket 27 and intermediate adhesive layer 31 may be formed from a commercially available adhesive film, such as acrylic transfer film adhesive, silicone transfer film, or adhesive films formed of acrylic, urethane, rubber, silicone, or other such adhesives. Alternatively, adhesive material may be printed, silk-screened, or otherwise deposited directly onto supporting layers. Various materials may be used to prepare the microfluidic interface mixing devices within the scope of the present invention. Suitable materials for use in the device may be selected to meet desired functional requirements of appropriate flexibility, rigidity, durability for the supporting layer and diaphragm layer, appropriate adhesion properties for the adhesive gasket material and intermediate adhesive layer, acceptable level of outgassing for all materials, and desired surface properties of hydrophilicity or hydrophobicity. It is possible for gasket 27 to be formed of a resilient, but non-adhesive or minimally adhesive material. In this case sealing of the gasket to the slide would be accomplished by clamping the device to the slide. The device configuration shown is one example of such an interface device, and functionally equivalent or similar structures can be formed from different numbers and configurations of layers.

Example 2

FIG. 3 depicts an alternative embodiment of the invention in which mixing bladders for generating pneumatic mixing are incorporated into a "hard shell" microarray interface device. This device is functionally similar to the device of FIG. 2, in that it includes a low aspect ratio reaction or hybridization chamber, like reaction chamber 3 in FIG. 1, having a

length and width defined by a perimeter wall 70 formed by an opening 69 in a gasket 71. The height of the chamber may be defined by the thickness of gasket 71. However, in other embodiments, not shown in the figures, the chamber height may be modified by setting gasket 71 into a recess in top layer 73, or by recessing the portion of top layer 73 that defines the upper surface of the chamber. Inlet port 5 and outlet port 7 allow fluid to be loaded into and removed from the reaction chamber defined by perimeter wall 70. Note that inlet port 5 and outlet port 7 align with holes 10 and 12 in diaphragm layer 33. Two mixing bladders defined by recesses 77 and 79 are located adjacent the reaction chamber to drive mixing. The device of FIG. 3 differs from the adhesive laminate device of FIG. 2 in that the device is sealed to the microarray slide to form a hybridization chamber by means of a resilient, but preferably not adhesive, gasket 71, whereas the device of FIG. 2 preferably utilizes an adhesive gasket. Sealing is accomplished by pressing the lid against slide 25 with a clamp (not shown). One function of the solid lid is thus to distribute the clamping pressure uniformly over the gasket to form a good seal with the slide surface. In addition, the device of FIG. 3 is formed from layers of rigid material that are sufficiently thick that structures can be formed within the layer thickness, while the device of FIG. 2 is formed from multiple thin, flexible layers. The device of FIG. 3 may include a gasket formed of various resilient or plastically deformable materials, including plastics, waxes, and elastomers. The rigid portions of the device of FIG. 3 may be formed of plastics such as polycarbonate, PTFE, FEP, PFA, PET, PMMA , silicon-based materials, glass, metals, or other rigid or semi-rigid materials.

As shown in FIG. 3, in order to form mixing bladders, the bottom surface 75 of top layer 73 has recesses 77 and 79 built into it in locations near inlet port 5 and outlet port 7. These recesses are enclosed by diaphragm layer 33, a thin film or layer of polymer attached to bottom surface 75 of top layer 73, to form mixing bladders that can be inflated or deflated by the application of pressure or vacuum, respectively. Mixing bladders 13 and 15, defined by recesses 77 and 79, can be seen in FIG. 4, which depicts the device of FIG. 3 in cross section. Referring back to FIG. 3, recesses 77 and 79 are accessed by channels 81 and 83, respectively, each of which connects to a recess at one end, and to a tube fitting, 85 or 87, at the other end. Tube fittings are used for attaching mixing bladders 13 and 15, respectively, to air lines from the pressure/vacuum source driving the pneumatic pumping bladders. In the example of FIG. 3, tube fittings are located at one end of the device, recessed into a finger tab 89 for protection, and connected to the channels via cross-drilled holes. Tube fittings may be placed in other locations, as well.

Use of the devices depicted in FIGS. 2-4 is as follows. The microarray user starts with a microarray slide which has been prehybridized and dried using standard protocols. FIG. 5 depicts an adhesive laminate microarray interface device 1, as shown in FIG. 2, which is adhered to slide 25 to form a hybridization chamber 3 on the surface of the slide. The hybridization chamber 3 is then filled with hybridization solution through inlet port 5. Air escapes via outlet port 7 as solution enters hybridization chamber 3. Following addition of hybridization solution, the inlet port 5 and outlet port 7 are preferably sealed, for example with adhesive tape tabs 6 as shown in FIG. 1, with plugs, or with other types of seals. A pressure source is connected to air channels 49 and 51, and air is pumped into and out of mixing bladders 13 and 15 in an appropriate frequency pattern to move the solution back and forth within the hybridization chamber 3. Heating may be provided as required to allow hybridization to take place, either by an external heater or by a heating element built into the device. Following hybridization, the slide may be subjected to a preliminary rinse by injecting wash solution into inlet port 5, followed by subsequent washes after the interface device is disassembled from the slide.

Pressure and Vacuum Sources.

Various types of pressure sources may be used to inflate and deflate mixing bladders in the inventive device. To simultaneously apply positive pressure to one bladder while applying negative pressure (vacuum) to the other bladder, a reciprocating pressure source 91, as illustrated in FIG. 1 in schematic form, can be used. Such a pressure source can be formed simply, for example, by securing two syringe pumps in fixed relationship to each other, and mechanically linking their plungers so that as one is pushed in the other is pulled out, to produce changes in volume that are of equal magnitude but opposite sign. Alternating positive and negative pressures can be generated by simply moving the linked plungers back and forth. One particular advantage of this arrangement is that the decrease in volume of the first bladder will be the same as the increase in volume of second bladder, so that no increase or decrease in pressure is produced in the fluid inside the hybridization chamber, which remains at substantially a constant volume.

Various types of pumps can be used to generate positive and negative pressures. In some cases, the action of the pump may not be readily reversed, so valves may be used to connect the mixing bladders alternately to the positive and negative pressure ends of the pump. The positive or negative connections of a pump may be connected to one or more manifolds which in turn are connected to multiple mixing bladders on one or multiple devices. The inventors have found that a 12 VDC air compressor is sufficient to drive

mixing in four devices of the type illustrated in FIG. 2 simultaneously. FIG. 6 depicts a system in which two manifolds 93 and 95 are alternately connected via valves 97 and 99 to first end 18 or second end 20 of pressure source 91, to provide positive and negative pressures alternately to mixing bladders 13 and 15 in multiple devices 1. Two 12 VDC 3-way solenoid valves are used as valves 97 and 99 in FIG. 6 to switch the manifolds 93 and 95 between the inlet and outlet ends of a 12 VDC air compressor used as pressure source 91. It is also possible to utilize separate pressure and vacuum sources calibrated to the appropriate signal amplitudes. Various other mechanisms for supplying positive and negative pressures may be devised for use in the present invention, and the invention is not limited to any specific pressure sources.

In the presently preferred embodiment of the invention, mixing bladders are driven by positive and negative pressures of equal magnitude but opposite sign. However, in theory it is only necessary that some sort of pressure differential be applied to generate mixing, and the practice of the invention is not limited to the use of balanced positive and negative pressures. For example, it would be possible to utilize two positive pressures of different magnitudes, two negative pressures of different magnitudes, or positive and negative pressures of different magnitudes. In practice, of course, the ability of the device to withstand positive or negative pressures while maintaining its structural integrity may limit the pressures that may be used to generate mixing.

Although it is preferred to pump air into and out of the bladders to drive fluid mixing, it would also be possible to pump another gas or a liquid into and out of the bladders to provide pumping, and this possibility is considered to fall within the scope of the invention. Moreover, mixing has been described in connection with systems that use two bladders, with one or both being actively inflated or deflated, but smaller or larger numbers of bladders may be used to provide mixing as well, being inflated or deflated in various patterns, and are considered to fall within the scope of the present invention.

In the presently preferred embodiment of the invention, the slide and attached microarray interface device are used in connection with an instrument that provides both heating and pressurized air. The instrument includes a hot block or slide warmer that holds multiple slides, and maintains the slides, interface devices, and hybridization chamber contents at the desired temperature during processing of the slide. The instrument preferably includes a light-proof thermal insulating cover that protects the labeled target from photo bleaching during prolonged incubations and maintains proper hybridization temperature. The instrument also includes one or more air manifolds to allow a single

reciprocating air pump, or other pressure and/or vacuum source, to supply alternating positive and negative pressures to multiple microarray interface device units, as depicted in schematic form in FIG. 6.

FIG. 7 is a cross sectional view of the device shown in FIG. 1, 2 and 5 in combination with an instrument. The base portion of the instrument is indicated at reference number 48. A microarray slide 25 with attached microarray interface device 1 fits into recess 60 in instrument base 48. Air lines 54 and 56 from a pump manifold in instrument base 48 are connected to the openings 53 and 55 of air channels 49 and 51 in the microarray interface device 1 by aligning the openings of air channels 53 and 55 with the openings of the air lines 54 and 56. Air line openings 54 and 56 are fitted with O-rings 50 and 52, such that a face seal can be formed by clamping interface device 1 against O-rings 50 and 52. Clamp 58, which clamps interface device 1 against O-rings 50 and 52 in instrument base 48, is preferably a part of the instrument and attached to instrument base 48.

A variety of different fittings may be used to connect a source of pressurized air (or other gas or liquid) to mixing bladders in a device that incorporates pneumatic mixing. For example, if air channels connecting to mixing bladders simply lead to openings in a smooth exterior surface of the microarray interface device, air line connectors that connect to an air line from a pressure source may be attached to air channel openings by clamping to form a face seal, as shown, or via double-sided adhesive disks. In other embodiments of the invention, air channel openings may be provided with push-to-connect fittings, such as the simple barbed fitting 84 shown in cross section in FIG. 8, over which air tubing 86 is pressed to make the connection. Alternatively, various types of quick-connect fittings may be provided, which automatically seal the tubing when the fittings are disconnected. Standard Luer taper fittings, with or without automatic sealing capacity, are further examples. The foregoing are merely exemplary, and the invention is not limited to any particular type of air channel connections.

In the presently preferred method for pneumatic mixing, pressure is alternately increased in a first bladder and simultaneously decreased in the second bladder, and then decreased in the first bladder and simultaneously increased in the second bladder. Because of the small volume of the hybridization chamber, only very small deflections of the bladders are necessary to cause displacement of the fluid in the hybridization chamber. If a first bladder is inflated while a second bladder is evacuated by the same amount, there is a net movement of the fluid in the hybridization chamber in the direction of the second bladder, to fill the volume made available by the evacuation of the second bladder. When

the procedure is reversed, the fluid in the hybridization chamber is made to flow in the opposite direction, toward the first bladder. It is preferred that the bladders are formed at either end of the hybridization chamber, separated by a region at least equal in area to the diaphragm regions, to provide better mixing. Moreover, it is also preferred that the bladders do not overlie the "active" region of the slide, on which the microarray is spotted, since it is possible that the bladder could touch the surface of the slide when inflated, and it is thought that this could disrupt any spots in the microarray that were contacted.

In the embodiment of the invention depicted in FIG. 2, the chamber has a volume of about 38 µl, height of 25 µm, length of approximately 67 mm and width of 20 mm. Two mixing bladders are provided, one at each end of the chamber. The area of each diaphragm is about 70 mm^2, and the height of each bladder is 25 µm, so that total volume of each bladder (when the diaphragm is at its rest or neutral position) is about 1.75 µl. The total volume of fluid displaced by each bladder, as the diaphragm is moved from the fully deflated position (withdrawn fully into the opening) to the fully inflated position (extending fully into the reaction chamber in the region below the bladder) is thus approximately 3.5 µl. Thus, inflation of a diaphragm causes about 9% of the volume of the chamber to be displaced. The amount of time that it takes for the contents of the chamber to be completely mixed is dependent on the percent of the chamber volume displaced by the diaphragm. It has been found that with 9% of the volume displaced by each bladder and a 8 second mixing cycle (alternating 4 seconds of positive pressure and 4 seconds of negative pressure on each bladder, with the bladders being 180° out of phase with each other), complete mixing is obtained in about 1 hour. Mixing is assessed by visual observation of the time at which uniform color is obtained in a chamber loaded with equal volumes of standard solution, one of which contains bromophenol blue dye, in opposite ends of the chamber such that fluid front between the two solutions is initially at the center of the chamber. If the system is constructed generally as above, but the area of the mixing bladder diaphragm is larger so it displaces about 25% of the chamber volume, complete mixing may be achieved in several minutes; conversely, if the mixing bladder diaphragm is reduced, the time to obtain mixing is increased. It is preferred that mixing bladders displace between about 0.1% and about 50% of the chamber volume, and more preferably between about 1% and about 30% of the chamber volume. It should be noted that the relative height of the chamber, diaphragm dimensions, and diaphragm material should preferably be selected such that when the mixing bladder is inflated, the diaphragm can be displaced into the chamber by a significant proportion of the chamber height, to displace a significant

percentage of the fluid in the region of the chamber below the mixing bladder. A number of factors besides the volume displaced by mixing bladder inflation influence mixing. These include the rate at which mixing bladders are inflated and deflated, the frequency of mixing bladder inflation, and the flexibility of the interface device lid. It has been found that switching alternately between applying positive and negative pressures every 10 seconds, for a cycle time of 20 seconds, is effective for achieving mixing. It has been observed that the fluid front moves about 10 mm per mixing cycle with this setup. If the cycle time is reduced much below 5 seconds, mixing performance decreases. It has been found that if the mixing cycle time is reduced to 5 seconds, minimal mixing effect is obtained, presumably because the viscosity of the DNA-containing solution is high enough that little movement of fluid is produced before the pressure is switched and the direction of movement is reversed. If the cycle time is increased, mixing may still be obtained; e.g., even cycle times of up to about three hours may produce a mixing effect. However, cycle times that are less than one minute (but not so short that viscosity limits fluid movement) are considered to be preferable. It should be noted that for hybridization reactions, it is not necessarily required that the solution be fully mixed early on in the hybridization process, since even modest movement of solution within the reaction chamber is sufficient to enhance the signal obtained from the hybridization reaction.

Pneumatically actuated microfluidic interface mixing devices within the scope of the present invention produce agitation and mixing of fluid within low aspect ratio microfluidic chambers, such as hybridization chambers, through the use of pneumatically actuated mixing elements. The most basic approach is to pressurize and depressurize two mixing bladders, alternately and in opposition, as described above. A number of other pneumatic mixing schemes may be used, as well.

One alternative approach, illustrated in FIG. 9, is to leave one mixing bladder, which will be referred to as passive mixing bladder 94, open to the atmosphere via vent 104, rather than connecting it to a pressure/vacuum source. The result is that the associated passive diaphragm region 90 is free to deflect outward and inward, respectively, as pressure in reaction chamber 3 increases and decreases when the other mixing bladder, referred to here as active mixing bladder 96, is actively pressurized and depressurized by pressure/vacuum source 91. As active mixing bladder 96 is inflated, it causes active diaphragm region 92 to deflect inward towards reaction chamber 3, which increases the pressure in reaction chamber 3 forcing the passive diaphragm region 90 to be deflected outward slightly with respect to reaction chamber 3, allowing fluid to move in the direction of passive diaphragm

90. When active mixing bladder 96 is deflated, active diaphragm region 92 deflects outward in relation to reaction chamber 3, the pressure within reaction chamber 3 decreases, causing passive mixing bladder 94 to expand passively, so that fluid moves in the direction of active mixing bladder 96. A variant (not shown) of the system illustrated in FIG. 9 is to omit passive mixing bladder 94 and vent 104 entirely, and allow one or both of inlet port 5 or outlet port 7, or another separately formed vent structure to function as a vent. The vent(s) must be sufficiently long that fluid displaced by the inflation of active mixing bladder 96 can move up and down within the vent, in communication with the atmosphere or with an enclosed air space of sufficient volume, but contained within the vent.

It is preferred that the mixing bladders are not positioned directly over spots in a microarray. However, in some cases the spotted array 117 may be positioned close to the edge of the slide 25, as depicted in FIG. 10. The gasket 27 may be modified to accommodate the spotted microarray 117, by squaring off opening 115 on end 103. Fluid is loaded into the reaction chamber defined by opening 115 via an inlet port defined by holes 121, 122, and 123 in top layer 29, intermediate adhesive layer 31, and diaphragm layer 33, respectively. As fluid is loaded, air escapes via three outlet ports defined by holes 125a, 125b, and 125c in top layer 29; 126a, 126b, and 126c in intermediate adhesive layer 31; and 127a, 127b, and 127c in diaphragm layer 33. Note that opening 115 in gasket 27 tapers to three outlet regions that align with holes 127a, 127b and 127c. The tapered outlet regions "funnel" air toward holes127a, 127b and 127c, to prevent air bubbles from being trapped at end 103, as would be likely to happen if opening 115 comprised a straight edge parallel to the edge of gasket 27 at end 103.

In the interface device of FIG. 10, there is no space for a mixing bladder at end 103 of the hybridization chamber, except over the array spots. To prevent the mixing bladder from contacting the spotted array 117 when it is inflated, which could potentially damage to the contacted spots, the usual single large mixing bladder is replaced by several (in this case two) narrower passive mixing bladders formed by openings 100 and 102 in intermediate adhesive layer 31. The diaphragm regions of the passive mixing bladders are not actively driven by reciprocating air pressure, but deflect in and out in response to changes in pressure generated within the reaction chamber by the active mixing bladder defined by opening 47 at the opposite end of the device. The smaller, passive deflection of multiple passive diaphragms will be less likely to disrupt the microarray spots. The active mixing bladder is connected to a pressure source (not shown) via air channel 119. The passive mixing bladders defined by openings 100 and 102 are connected to each other by channel

105 and to the atmosphere by vent 104 so that they can move in and out freely to equalize the chamber volume and pressure as the active bladder is pressurized and depressurized.

Another approach to mixing is to alternately pressurize each mixing bladder in turn, while allowing the opposite bladder to be open to the atmosphere. The diaphragm of the mixing bladder that is open to the atmosphere will then be passively deflected by increased pressure in the fluid in the hybridization chamber when pressure is applied to the other bladder. FIG. 11 illustrates a system configuration adapted for this approach. Valves 130 and 132 connect line 134 from pressure source 91 and line 136, which is vented to the atmosphere 94, alternately to line 17 connection to mixing bladder 13 and line 19 connecting to mixing bladder 15. Various types of valves can be used to switch the mixing bladders between connection to atmosphere 94 and pressure source 91.

Finally, it is also possible to apply a vacuum or negative pressure intermittently to either one or both of the bladders, while the opposite bladder is vented to the atmosphere and thus free to passively expand, to produce mixing. This approach requires that the system be configured as shown in FIG. 9 or FIG. 11, with pressure source 91 producing only negative, rather than positive and negative, pressure.

It is presently preferred that reciprocating pumping action be used, so that reaction chamber pressure and volume remain substantially constant during pumping. It is thought that, in the case of the adhesive laminate interface device, not only the passive diaphragm but also the entire relatively flexible interface device will tend to deflect if one bladder is actively pressurized but the other is not simultaneously depressurized, which may absorb the mixing force and lead to less effective mixing. Moreover, if the passive deflection of the diaphragms is not sufficient to relieve the pressure in the reaction chamber, the increased pressure could lead to separation of the lid from the slide, and hence leaking of fluid from the reaction chamber. However, various other pumping actions, including but not limited to those described herein, may be used instead and are considered to fall within the scope of the invention.

Interestingly, the flexible nature of the adhesive laminate interface device depicted in FIG. 2 has been observed to respond to alternate inflation and deflation of mixing bladders in a manner that enhances the mixing effect. Because DNA solutions used in hybridization reactions are fairly viscous, when a quantity of solution is displaced by the inflation of a mixing bladder, the displaced fluid tends to cause the interface device to bulge upward in the area adjacent the mixing bladder. The interface device bulges upward to a greater extent in the center than at the periphery of the chamber, because at the periphery of

the chamber the device is adhered to the slide with the adhesive gasket. Conversely, when a mixing bladder is deflated, as fluid is drawn into the region under the mixing bladder, the interface device adjacent the mixing bladder tends to be pulled slightly downward in the central region, but not at the periphery of the chamber, where it is supported by the gasket. The distance between the interface device and the slide in the vicinity of a mixing bladder is greater in the central region of the reaction chamber than at the periphery when fluid is being driven away from the mixing bladder, and it is greater at the periphery than at the central region when fluid is being drawn toward the mixing bladder. Fluid flow will be greatest in the regions where the distance between the slide and the interface device is largest. As a consequence, when an individual bladder is alternately inflated and deflated, a circulating fluid movement pattern is generated, and when both bladders are alternately inflated and deflated, two circulating patterns are generated which interact to provide enhanced mixing compared to what would be obtained if the inflation and deflation of bladders simply caused the fluid to move back and forth with a uniform fluid front in the reaction chamber.

Although in the situation described above, the difference in chamber height between the central region and periphery of the reaction chamber is produced in response to the inflation and deflation of mixing bladders used to produce fluid movement, it may also be possible to provide separate mechanisms for driving fluid movement and modifying chamber height. If the reaction chamber aside from the mixing bladders is sufficiently rigid that it does not flex passively in response to fluid movement, various displacement mechanisms could be used to displace the central region of the interface device to increase or decrease the height of the reaction chamber in its central region. Such displacement mechanisms could include mechanical linkages attached to the wall of the interface device to cause displacement. Another displacement mechanism would be to form part or the entire wall of the chamber from a material that would change its dimensions in response to a change in a parameter such as electrical field, temperature, moisture, etc. By coordinating inward and outward displacement of the reaction chamber wall with pumping of fluid back and forth within the chamber (by pumping mechanisms such as pneumatic mixing bladders or other mechanisms as may be known to those of ordinary skill in the art), circulating fluid patterns may be generated within the reaction chamber.

The pneumatic mixing structures according to the present invention are not restricted to use in devices that form reaction or hybridization chambers on slides, and may also be incorporated into a variety of other microfluidic devices. FIG. 12 depicts a generic

microfluidic device 137 formed from multiple layers 138, 140, 142, 144, and 146 of rigid material, secured together. Selected regions of the layers are voids that form a number of chambers and channels within device 137. It is contemplated that the device of FIG. 12 could be formed in various relatively rigid materials, e.g. various types of plastics, glass, or other silicon-based materials. Layers formed of these materials may be secured together by adhesives, thermal bonding, and other methods.

The structure shown in FIG. 12 may be just a portion of a larger device, which may include various other types of microfluidic circuitry, for example. The structure of FIG. 12 includes a low aspect ratio chamber 148 having inlet channel 150 and outlet channel 152. FIG. 12 is a cross section taken through the height, h, of the low aspect ratio chamber. The height h and length l are not necessarily depicted to scale. Two mixing bladders, 154 and 156, are formed adjacent low aspect ratio reaction chamber 148. Air (or another gas or liquid) enters and exits mixing bladder 154 via air channel 158, and enters and exits mixing bladder 156 via air channel 162, to cause inward and outward deflection of diaphragm regions 160 and 164, respectively. In this example, diaphragm region 160 is formed integrally with layer 140, in which mixing bladder 154 is formed, and diaphragm region 164 is formed integrally with layer 144, in which mixing bladder 156 is formed. Methods of forming thin diaphragms in polymeric or silicon-based substrates, which may include molding, cutting, machining, printing methods, etching, vapor deposition, embossing, and so forth, are known to those of ordinary skill in the art. It would also be possible to form a diaphragm in a microfluidic device similar to that shown in FIG. 12 (i.e., formed of multiple relatively rigid layers and designed to be self-contained, rather than forming a reaction chamber by combination with a slide or other substrate), by including a flexible diaphragm layer as used in the embodiments of FIGS. 1-4 between rigid layers forming the structure of the device. The diaphragm layer could be attached by laminating, adhesive, ultrasonic bonding, and so forth. Conversely, a device of FIGS. 3 and 4, which includes several layers of relatively rigid material, could include diaphragms formed integrally with one of the layers in the manner depicted in FIG. 12. FIG. 12 also illustrates the possibility of forming mixing bladders on opposite sides of the reaction chamber 148 in which mixing occurs, and illustrates additional alternative configurations for the channels leading to the mixing bladders and reaction chamber.

Another variation of the present invention is to use pneumatic mixing bladders to generate mixing in multiple reaction chambers simultaneously. FIG. 13 illustrates, in an exploded view, a device having multiple reaction chambers with mixing bladders overlying

the ends of the chambers to provide mixing in all chambers. Multiple parallel chambers are formed on a microarray slide 25 (or other substrate) by using a gasket 172 having multiple parallel slots 101, which when sealed between microarray slide 25 and diaphragm layer 187 define multiple elongated chambers. Such elongated chambers may have lengths comparable to those described for the larger single chambers illustrated in FIGS. 2 and 3, but widths ranging from several hundred microns to several millimeters. Multiple inlet ports are formed by holes 182, 183, and 184 in top layer 166, intermediate adhesive layer 168, and diaphragm layer 170, respectively, which allow fluid to be delivered to the reaction chambers. Multiple outlet ports formed by holes 185, 186, and 187 in top layer 166, intermediate adhesive layer 168, and diaphragm layer 170, respectively, allow fluid to exit the reaction chambers. It is not necessary to provide two separate mixing bladders to each chamber, since it is possible for a single mixing bladder to overlap and produce fluid displacement in multiple chambers simultaneously. Thus, just two openings 174 and 176 defining mixing bladders can be used, as shown in FIG. 13. Although the reaction chambers depicted in FIG. 13 are straight and parallel, this does not necessarily have to be the case, providing that a mixing bladder can be located at or near the end of each chamber. For example, chambers may have curved or serpentine configurations, or any other configurations that permit mixing bladders to be positioned at opposite ends to generate fluid movement therein. FIG. 13 depicts three parallel reaction chambers. The invention is not limited to any particular number of reaction chambers; one, two, three, or more reaction chambers may be used. A design which utilizes a pair of mixing bladders to generate mixing in multiple reaction chambers will be practical for designs in which larger numbers of reaction chambers are used. If only a small number of reaction chambers are used (e.g., two or three), it would also be possible to have separate pairs of mixing bladders for each reaction chamber, in which case reaction chambers need not be arranged with their ends adjacent each other.

In the embodiments of the invention depicted in FIGS. 1–4, the diaphragms displaced by the mixing bladders are part of a continuous diaphragm layer formed of a thin, flexible sheet material adhered to an adjacent layer in which recesses that define the mixing bladders are formed. In FIG. 12, diaphragms are formed integrally with the material in which the recesses are formed. Other methods of forming diaphragms and/or mixing bladders may also be devised. For example, rather than using a continuous sheet of diaphragm material that forms diaphragms over two, or possibly more, recesses, it would be possible to use small individually formed diaphragms that each cover a single recess. Still

another approach is to construct mixing bladders as independent, balloon-like structures that can be secured to the interior of the chamber. FIG. 14 depicts two balloon-like mixing bladders 113 secured to wall 116 of reaction chamber 118 in device 120. Mixing bladders 113 are secured to wall 116 with an adhesive 112, for example. Each mixing bladder 113 is pressurized and depressurized via an air tube 114. Solid and dashed lines distinguish the inflated and uninflated dimensions of mixing bladders 113 in FIG. 14. Balloon-like structures 113 could be formed as one-piece molded structures or by bonding together two layers of resilient sheet material. Air tubes 114 may be formed integrally with mixing bladders 113, formed separately and then attached to mixing bladders 113, or formed within the structure of device 120 in the same manner as other channels for conveying air or liquid.

The present invention is a method and system for generating fluid mixing within a low aspect ratio chamber by deflecting two or more regions of the chamber wall inward and outward, to move fluid back and forth while maintaining a substantially constant chamber volume. In the preferred embodiment of the invention, flexible regions of the chamber wall (diaphragms) are moved inward and outward by increasing and or decreasing pressure in mixing bladders adjacent the diaphragm regions. However, it will be appreciated that deflection of flexible wall regions may be produced by the application of pressure to the chamber wall by other methods. For example, rather than inflating mixing bladders to deflect diaphragms, it would be possible to apply mechanical force to deflect diaphragms. Moreover, in a device such as the adhesive laminate microarray interface device 1 depicted in FIG. 2, in which the entire device is relatively flexible, it may not be necessary to provide discrete regions of the device having higher flexibility. Rather, mechanical pressure may be applied alternately to selected areas (e.g., at either end) of a generally flexible chamber wall to move fluid back and forth within the chamber. Pressure could be applied for example, by mechanical feet 107 and 109 that alternately depress and release opposite ends of the surface of device 1, as shown in FIG. 15. Mechanical feet 107 and 109 may be driven by shafts 108 and 110, which are moved up and down in reciprocal fashion by crank shaft 189, or by various other mechanisms for generating reciprocating motion, as are known to those of ordinary skill in the art. Alternatively, as shown in FIGS. 16A and 16B, a brayer-type device 111 may rolled or slid back and forth across the surface of the device 1 to move fluid back and forth within reaction chamber 3. In the embodiments of FIGS. 15, 16A and 16B, it is preferred that device 1 is depressed in a controlled manner such that it does not press against the surface of slide 25 and damage reactants present on the surface of slide 25.

The present invention is described and disclosed in connection with a number of examples. However, the scope of the invention is not limited to the specific examples provided herein, but is intended to include various modifications as may be devised by those of ordinary skill in the art, and is defined by the claims appended hereto.

CLAIMS:

1. A microfluidic device comprising:

a. at least one low aspect ratio chamber comprising:

i. two substantially parallel main walls;

ii. a perimeter wall forming a boundary of said chamber and defining a length and width of said chamber, said chamber being further bounded by said two main walls, the distance between said main walls being small with respect to the length of said chamber; and

iii. at least two flexible diaphragm regions located in said parallel main walls at opposite ends of said at least one chamber and adapted to flex inward and outward with respect to said chamber; and

b. at least one inlet port through which fluid may be introduced into said at least one low aspect ratio chamber, wherein said at least one inlet port is sealable to maintain a substantially constant volume within said chamber.

2. The microfluidic device of claim 1, wherein said microfluidic device comprises a plurality of low aspect ratio chambers, wherein each of said low aspect ratio chambers comprises:

a. two substantially parallel main walls;

b. a perimeter wall forming a boundary of said chamber and defining a length and width of said chamber, said chamber being further bounded by said two main walls, the distance between said main walls being small with respect to the length of said chamber; and

c. at least two flexible diaphragm regions located in said parallel main walls at opposite ends of said at least one chamber.

3. The microfluidic device of claim 2, wherein each of said at least two flexible diaphragm regions is located adjacent to a bladder connected to an external pressure source and adapted to flex inward or outward in response to pressure changes in said bladder, and wherein one said bladder is located adjacent to diaphragm regions of multiple chambers.

4. The microfluidic device of claim 1, wherein said flexible diaphragm regions are separated by a region at least equal in area to one of said diaphragm regions.

5. The microfluidic device of claim 1, wherein said flexible diaphragm regions displace between about 0.1% and about 50% of the volume of said chamber.

6. The microfluidic device of claim 1, wherein said flexible diaphragm regions displace between about 1% and about 30% of the volume of said chamber.

7. The microfluidic device of claim 1, wherein said microfluidic device further comprises a chemically active region on at least one of said parallel main walls, and wherein said flexible diaphragm regions are positioned so that they do not overly said chemically active region.

8. The microfluidic device of claim 2, wherein the ratio of the distance between said main walls to the length of said chamber is between about 1:300 and about 1:10,000.

9. The microfluidic device of claim 2, wherein the ratio of the distance between said main walls to the length of said chamber is between about 1:100 and about 1:1,000.

10. The microfluidic device of claim 2, wherein the distance between said main walls is between about 10 μm and about 50 μm.

11. The microfluidic device of claim 2, wherein the distance between said main walls is between about 15 μm and about 30 μm.

12. The microfluidic device of claim 2, wherein the distance between said main walls is between about 50 μm and about 300 μm.

13. The microfluidic device of claim 1, wherein said at least two flexible diaphragm regions are located at opposite ends of one of said main walls.

14. The microfluidic device of claim 1, wherein one flexible diaphragm region is located on one wall, and at least one other flexible diaphragm region is located on the other main wall.

15. The microfluidic device of claim 1, wherein each of said least two flexible diaphragm regions is located adjacent to a bladder connected to an external pressure source, and adapted to flex inward or outward in response to changes in pressure within said bladder.

16. The microfluidic device of claim 15, wherein said pressure source changes the pressure of a gas or gaseous mixture within said bladder.

17. The microfluidic device of claim 15, wherein said pressure source changes the pressure of a liquid within said bladder.

18. The microfluidic device of claim 1, wherein each of said at least two flexible diaphragm regions is adapted to flex inward or outward in response to mechanical pressure delivered by a mechanical actuator.

19. The microfluidic device of claim 18, wherein said mechanical actuator comprises a roller or a brayer.

20. The microfluidic device of claim 18, wherein said mechanical actuator comprises mechanical feet adapted to alternately apply and release pressure to said flexible diaphragm regions.

21. The microfluidic device of claim 1, wherein said device comprises a substantially rigid base material.

22. The microfluidic device of claim 21, wherein said diaphragm regions are formed integrally with said substantially rigid base material.

23. The microfluidic device of claim 21, wherein said diaphragm regions are formed by a method selected from molding, cutting, machining, printing methods, etching, vapor deposition, and embossing.

24. The microfluidic device of claim 21, wherein said diaphragm regions are formed from flexible sheet material adhered to said substantially rigid base material.

25. The microfluidic device of claim 1, wherein said device is formed from flexible material.

26. The microfluidic device of claim 25, wherein said diaphragm regions are formed by a method selected from molding, cutting, machining, printing methods, etching, vapor deposition, and embossing.

27. The microfluidic device of claim 25, wherein said diaphragm regions are formed from flexible sheet material adhered to said substantially rigid base material.

28. The microfluidic device of claim 1, further comprising at least one outlet port through which fluid may escape from said at least one low aspect ratio chamber, wherein said at least one outlet port is sealable to maintain a substantially constant volume within said chamber.

29. The microfluidic device of claim 1, further comprising a plurality of outlet ports through which fluid may escape from said at least one low aspect ratio chamber, wherein each said outlet port is sealable to maintain a substantially constant volume within said chamber

30. The microfluidic device of claim 29, wherein said chamber comprises a plurality of outlet regions tapering toward said outlet ports.

31. A reaction chamber forming device comprising an open low aspect ratio chamber adapted to be sealed against a substrate, said chamber comprising:

a. at least one substantially planar main wall, comprising two flexible diaphragm regions adapted to flex inward and outward with respect to said chamber in response to applied pressure;

b. a perimeter wall forming a boundary of said chamber and defining a length and width of said chamber, the height of said perimeter wall defining the height of said chamber, said height of said chamber being small with respect to the length of said chamber, said height of said chamber being small with respect to the length of said chamber;

c. at least one inlet port through which fluid may be introduced into said chamber;

d. at least one outlet port through which fluid may be removed or released from said chamber; and

e. a gasket adapted to reversibly seal said open low aspect ratio chamber to a planar surface of a substrate bearing a sample to form a closed low aspect ratio chamber containing said sample and having one wall formed by said surface of said substrate.

32. The microfluidic device of claim 31, wherein the height of said chamber is between about 10 μm and about 50 μm.

33. The microfluidic device of claim 31, wherein the height of said chamber is between about 15 μm and about 30 μm.

34. The microfluidic device of claim 31, wherein the height of said chamber is between about 50 μm and about 300 μm.

35. The reaction chamber forming device of claim 31, further comprising at least one bladder adjacent to said main wall, wherein said main wall is adapted to flex inward and outward with respect to said chamber in response to inflation and deflation of said bladder.

36. The reaction chamber forming device of claim 31, wherein said device comprises a substantially rigid base material.

37. The reaction chamber forming device of claim 36, wherein said diaphragm regions are formed integrally with said substantially rigid base material.

38. The reaction chamber forming device of claim 37, wherein said diaphragm regions are formed by a method selected from molding, cutting, machining, printing methods, etching, vapor deposition, and embossing.

39. The reaction chamber forming device of claim 37, wherein said diaphragm regions are formed from flexible sheet material adhered to said substantially rigid base material.

40. The reaction chamber forming device of claim 31, wherein said device comprises a base structure formed of flexible material.

41. The reaction chamber forming device of claim 40, wherein said diaphragm regions are formed by a method selected from molding, cutting, machining, printing methods, etching, vapor deposition, and embossing.

42. The reaction chamber forming device of claim 40, wherein said diaphragm regions are formed from flexible sheet material adhered to said base structure.

43. The reaction chamber forming device of claim 31, wherein said microfluidic device comprises a plurality of open low aspect ratio chambers, wherein each of said open low aspect ratio chambers comprises:

a. a substantially planar main wall;

b. a perimeter wall forming a boundary of said chamber and defining a length and width of said chamber, the height of said perimeter wall defining the height of said chamber, said height of said chamber being small with respect to the length of said chamber;

wherein said gasket comprises a plurality of openings formed therethrough, each opening corresponding to one said chamber.

44. The microfluidic device of claim 43, further comprising at least one bladder adjacent to the main walls of multiple said chambers, wherein said main walls are adapted to flex inward and outward with respect to said chambers in response to inflation and deflation of said bladder, wherein said at least one bladder is connected to an external pressure source and adapted to flex inward or outward with respect to said plurality of chambers in response to pressure changes in said bladder.

45. The microfluidic device of claim 31, wherein the ratio of the distance between said main walls to the length of said chamber is between about 1:30 and about 1:10,000.

46. The microfluidic device of claim 31, wherein the ratio of the distance between said main walls to the length of said chamber is between about 1:100 and about 1:1,000.

47. The microfluidic device of claim 31, wherein the ratio of the distance between said main walls to the length of said chamber is between about 1:200 and about 1:300.

48. The microfluidic device of claim 31, wherein said at least one outlet port is sealable to maintain a substantially constant volume within said chamber.

49. The microfluidic device of claim 31, comprising a plurality of outlet ports through which fluid may escape from said at least one low aspect ratio chamber, wherein each said outlet port is sealable to maintain a substantially constant volume within said chamber.

50. The microfluidic device of claim 49, wherein said chamber comprises a plurality of outlet regions tapering toward said outlet ports.

51. A fluid handling device comprising:

a. low aspect ratio chamber formed in a base structure;

b. at least one inlet through which fluid can be introduced into said low aspect ratio chamber, wherein said at least one inlet is sealable to maintain a substantially constant volume within said chamber;

c. a first mixing bladder located at a first end of said chamber;

d. a first channel communicating with said first mixing bladder;

e. a second mixing bladder located at a second end of said chamber; and

f. a second channel communicating with said second mixing bladder;

wherein said first and second mixing bladders may be alternately and reciprocally inflated and deflated to produce movement of fluid within said chamber while maintaining said chamber at said substantially constant volume.

52. The fluid handling device of claim 51, wherein said base structure is formed of flexible material.

53. The fluid handling device of claim 52, wherein said first and second mixing bladders are formed from a layer of a flexible sheet material secured over recesses formed in said base structure.

54. The fluid handling device of claim 52, wherein each of said first and second mixing bladders is formed from a separate diaphragm of a flexible sheet material secured over a recess formed in said base structure.

55. The fluid handling device of claim 52, wherein each of said first and second mixing bladders is formed from a balloon-like structure separately formed and secured within said chamber.

56. The fluid handling device of claim 51, wherein said base structure is formed of a rigid or semi-rigid material.

57. The fluid handling device of claim 56, wherein said first and second mixing bladders are formed from a layer of a flexible sheet material secured over recesses formed in said base structure.

58. The fluid handling device of claim 56, wherein each of said first and second mixing bladders is formed from a separate diaphragm of a flexible sheet material secured over a recess formed in said base structure.

59. The fluid handling device of claim 56, wherein each of said first and second mixing bladders is formed from a balloon-like structure separately formed and secured within said chamber.

60. The fluid handling device of claim 56, wherein said base structure is formed from at least two layers of rigid material secured together, and wherein each of said first and second mixing bladders comprises:

a. a diaphragm formed integrally with a layer of said base structure; and

b. a recess formed in a face of a layer of said base structure;

wherein said recess is enclosed by securing together two layers of said base structures.

61. The fluid handling device of claim 60, wherein said diaphragm is formed by molding, cutting, machining, printing methods, etching, vapor deposition, and embossing.

62. The fluid handling device of claim 51, wherein said first and second mixing bladders are inflated and deflated by gas pressure differentials transmitted via said first and second channels.

63. The fluid handling device of claim 51, wherein said first and second mixing bladders are inflated and deflated by liquid pressure differentials transmitted via said first and second channels.

64. A method of mixing fluid within a low aspect ratio chamber, said chamber comprising:

a. two substantially planar and substantially parallel main walls;

b. a perimeter wall forming a boundary of the low aspect ratio chamber and defining a length and width of said chamber, said chamber being further bounded by said two main walls, the distance between said main walls being small with respect to the length of said chamber; and

c. at least first and second flexible diaphragm regions located in at least one of said parallel main walls at opposite ends of said chamber;

d. at least first and second mixing bladders, one mixing bladder adjacent to each said flexible diaphragm region; and

e. an inlet port through which fluid may be introduced into said low aspect ratio chamber;

said method comprising the steps of:

i. loading a volume of fluid into said chamber via said inlet port;

ii. sealing said inlet port to retain said volume of fluid within said chamber;

iii. inflating said first mixing bladder to cause deflection of said first flexible diaphragm region into said chamber; and

iv. deflating said first mixing bladder to cause deflection of said first flexible diaphragm region out of said chamber;

wherein said volume of said chamber remains substantially constant during said steps of inflating and deflating said first mixing bladder.

65. The method of claim 64, wherein steps iii) and iv) are repeated one or more times, and wherein each repetition of steps iii) and iv) comprises one mixing cycle.

66. The method of claim 65, wherein each said mixing cycle has a cycle time of between about 5 seconds and about 3 hours.

67. The method of claim 65, wherein each said mixing cycle has a cycle time of between about 5 seconds and about 1 minute.

68. The method of claim 64, wherein said first mixing bladder is actively inflated and actively deflated.

69. The method of claim 64, wherein said first mixing bladder is actively inflated and passively deflated.

70. The method of claim 64, wherein said first mixing bladder is passively inflated and actively deflated.

71. The method of claim 64, comprising the further step of alternately deflating said second mixing bladder to cause deflection of said second flexible diaphragm region out of said chamber as said first mixing bladder is inflated, and inflating said second mixing bladder to cause deflection of a second flexible diaphragm region into said chamber as said first mixing bladder is deflated.

72. The method of claim 71, wherein said first and second mixing bladders are actively inflated and actively deflated.

73. The method of claim 71, wherein said first and second mixing bladders are actively inflated and passively deflated.

74. The method of claim 71, wherein said first and second mixing bladders are passively inflated and actively deflated.

75. The method of claim 71, wherein said first and second mixing bladders are connected to inlet and outlet ends of a common pressure source.

76. The method of claim 71, wherein said first and second mixing bladders are connected to separate pressure sources calibrated to produce equal and opposite pressures.

77. The method of claim 71, wherein each of said first and second mixing bladders is switched alternately between a positive pressure source and a negative pressure source through the use of a valve.

78. The method of claim 64, wherein said second mixing bladder is vented to the atmosphere, so that said second flexible diaphragm is permitted to passively deflect outward when said first flexible diaphragm is deflected inward, and passively deflect inward when said first flexible diaphragm is deflected outward.

79. The method of claim 64, wherein said first and second mixing bladders are alternately and reciprocally switched between a positive pressure source and atmospheric pressure.

80. The method of claim 64, wherein said first and second mixing bladders are alternately and reciprocally switched between a negative pressure source and atmospheric pressure.

81. The method of claim 64, wherein said first and second mixing bladders are alternately and reciprocally switched between a positive pressure source and a negative pressure source.

82. A fluid handling device comprising a low aspect ratio chamber, the low aspect ratio chamber comprising

a. a substantially planar first main wall;

b. a substantially planar second main wall positioned substantially parallel with respect to said first main wall; and

c. a perimeter wall bounding the region between said first and second main walls, the height of said perimeter wall defining a microscale distance between said first and second main walls that is significantly smaller than the length and width of said first and second main walls;

wherein edges of said first and second main walls are fixed with respect to said perimeter wall, wherein at least one said first and second main walls comprises a central wall region adapted to flex inward and outward with respect to said chamber, wherein inward flexing of said central wall region causes the distance between said first and second main walls to be less in the central wall region of said chamber than in a peripheral region adjacent said perimeter wall, and wherein outward flexing of said central wall central wall region causes the distance between said first and second main walls to be greater in said central wall region than in said peripheral region.

83. The device of claim 82, further comprising at least one pumping mechanism for pumping fluid back and forth alternately in a first direction and a second direction within said low aspect ratio chamber, wherein said at least one central wall region is flexed outward when fluid is driven in said first direction, causing fluid to move preferentially in the central region of said chamber, and wherein said at least one central wall region is flexed inward when fluid is driven in said second direction, causing fluid to move preferentially along the sides of said chamber, thereby producing circulating movement of said fluid within said chamber.

84. The device of claim 83, wherein said at least one central wall region is flexed inward and outward actively by a displacement mechanism.

85. The device of claim 83, wherein said at least one central wall region is flexed inward and outward passively by the force of the fluid as it is moved back and forth in said chamber by said pumping mechanism

86. A method of mixing fluid within a microfluidic device comprising a substantially fixed volume low aspect ratio chamber, wherein at least selected regions of the wall of said device adjacent said chamber can be moved inward or outward with respect to said chamber, the method comprising the steps of:

a. filling said chamber with fluid;

b. sealing said chamber to retain fluid within said chamber;

c. pumping said fluid in a first direction within said chamber;

d. deflecting a central wall region the wall of said device so that in at least a portion of said chamber, the height of said chamber is greater in the central wall region of said chamber than on the periphery of said chamber;

e. pumping said fluid in a second direction within said chamber;

f. deflecting a central wall region the wall of said device so that in at least a portion of said chamber, the height of said chamber is less in the central wall region of said chamber than on the periphery of said chamber;

g. repeating steps c. through f. to generate circulating fluid movement within said chamber.

87. A method of mixing fluid within a fluid-filled microfluidic device comprising a substantially fixed volume low aspect ratio chamber, wherein at least selected regions of the wall of said device adjacent said chamber can be moved inward or outward with respect to said chamber, the method comprising applying pressure alternately to movable wall regions on opposite ends of said chamber by a brayer, a roller, or mechanical feet.

88. A microfluidic device comprising:

a. at least one low aspect ratio chamber comprising:

i. two substantially parallel main walls;

ii. a perimeter wall forming a boundary of said chamber and defining a length and width of said chamber, said chamber being further

bounded by said two main walls, the distance between said main walls being small with respect to the length of said chamber;

iii. at least one flexible diaphragm region located in one of said parallel main walls adapted to flex inward and outward with respect to said chamber; and

iv. and least one vent, said vent being sufficiently long that fluid displaced by the inward and outward flexing of said at least one flexible diaphragm region can move up and down within said vent while remaining contained within said vent; and

b. at least one inlet port through which fluid may be introduced into said at least one low aspect ratio chamber.

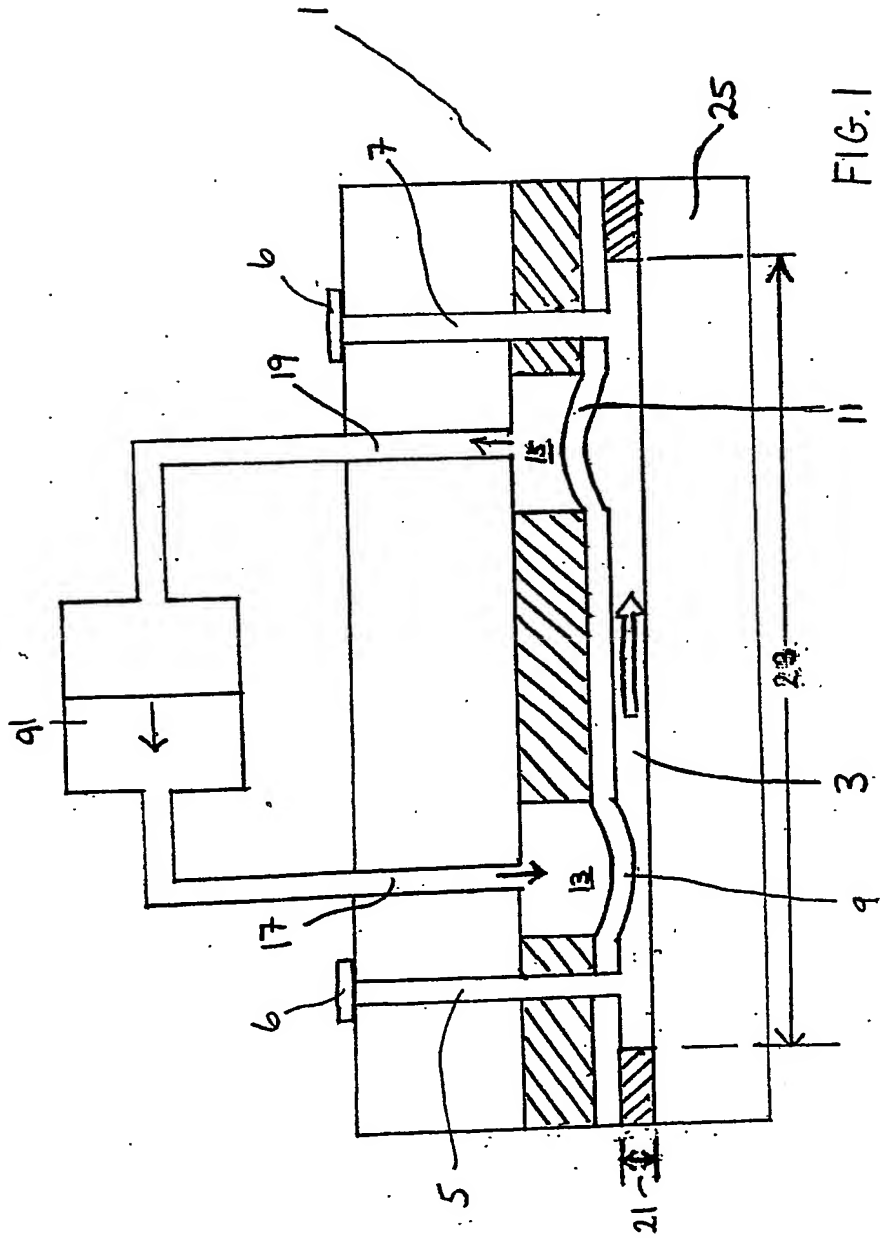


FIG. 1

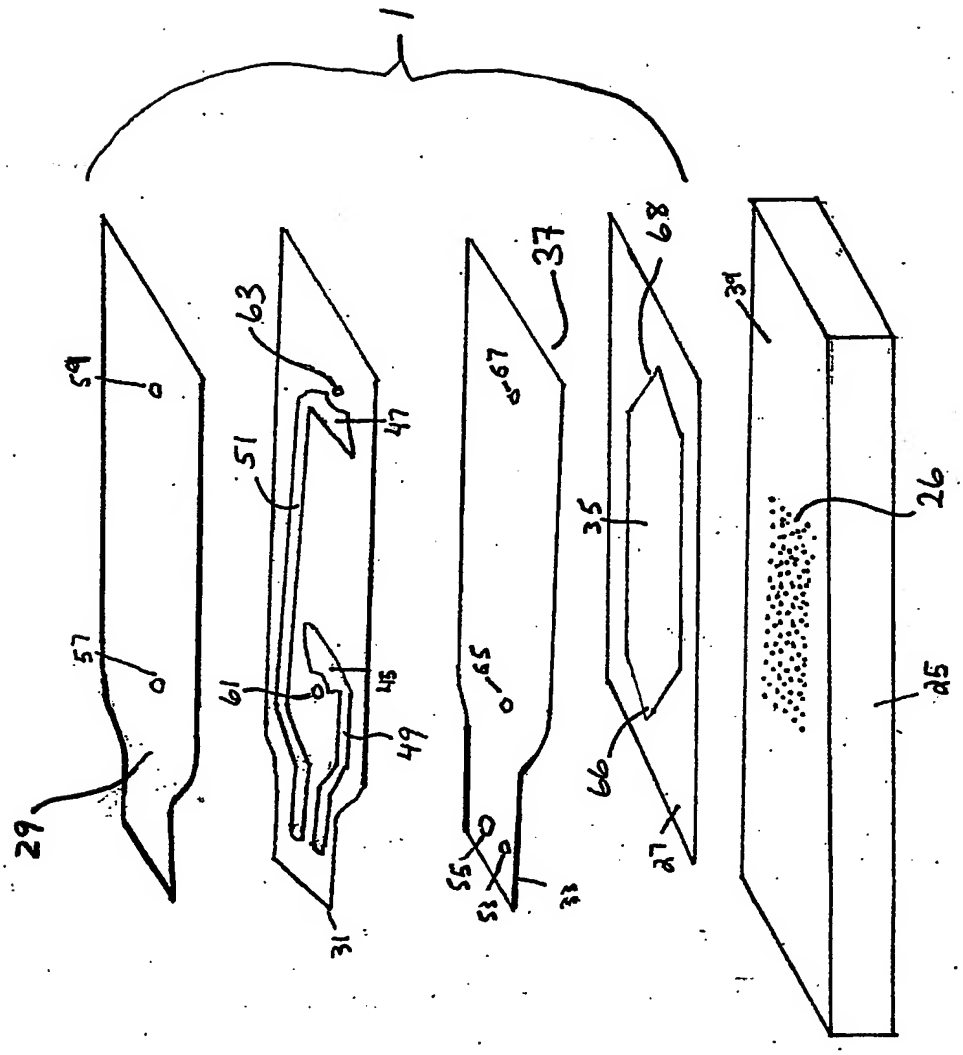


FIG.2

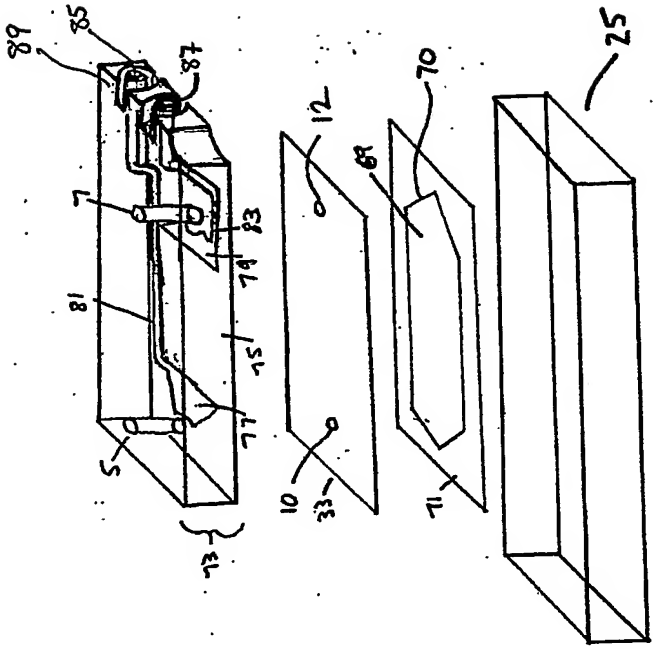


FIG.3

(WO 03015923A1 | >)

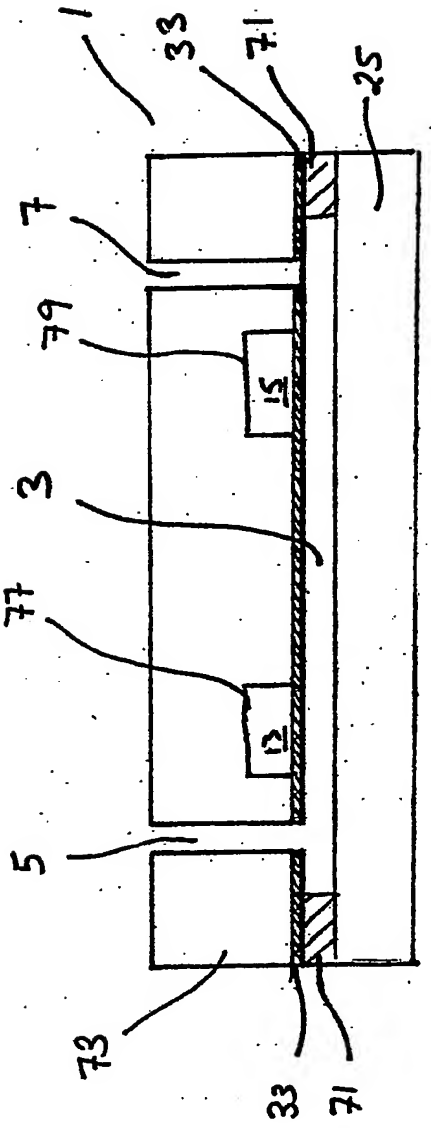
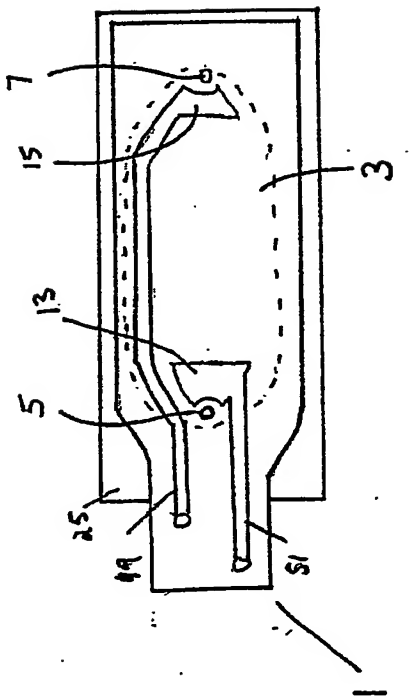


FIG. 4

FIG. 5



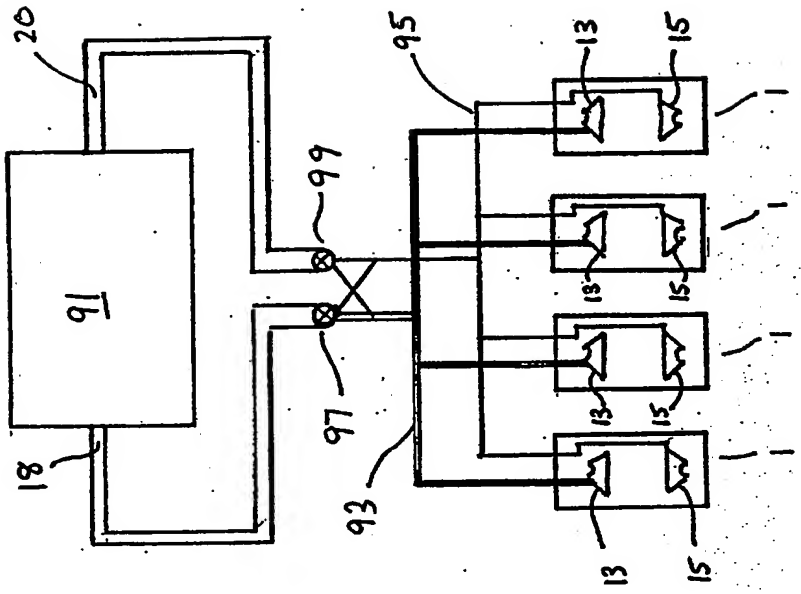


FIG. 6

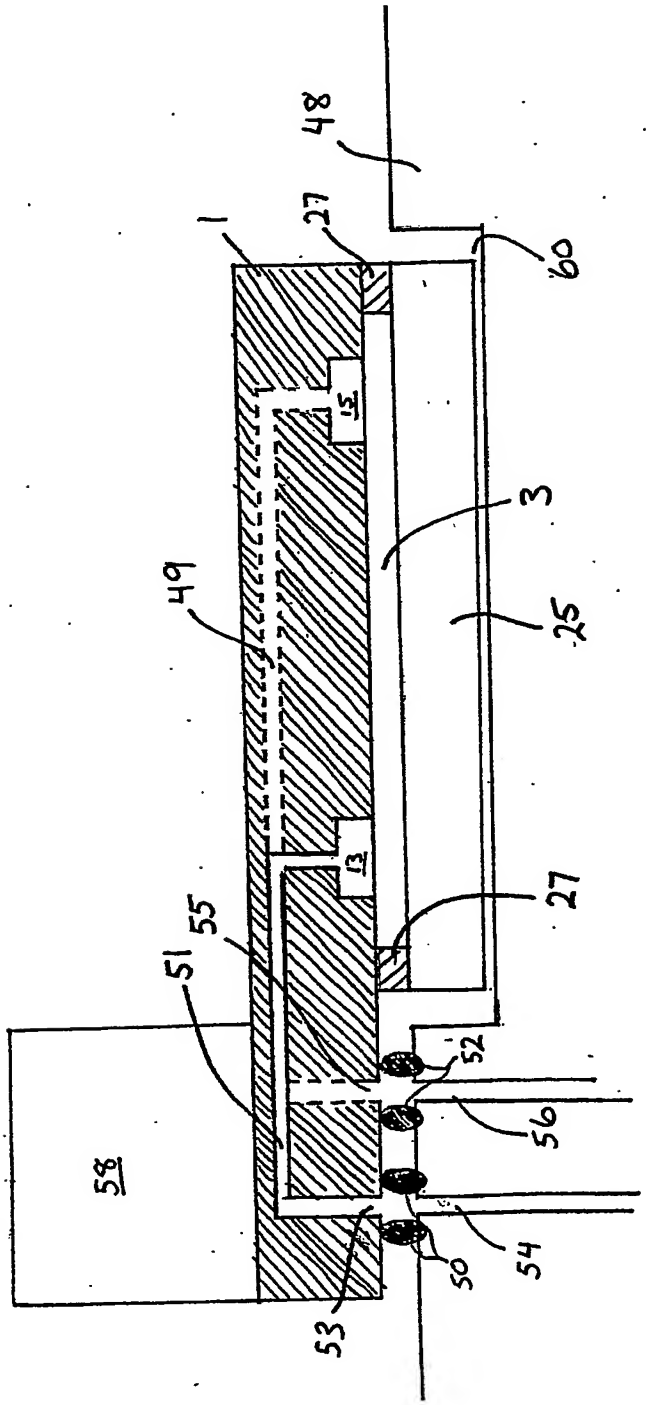


FIG. 7

FIG. 8

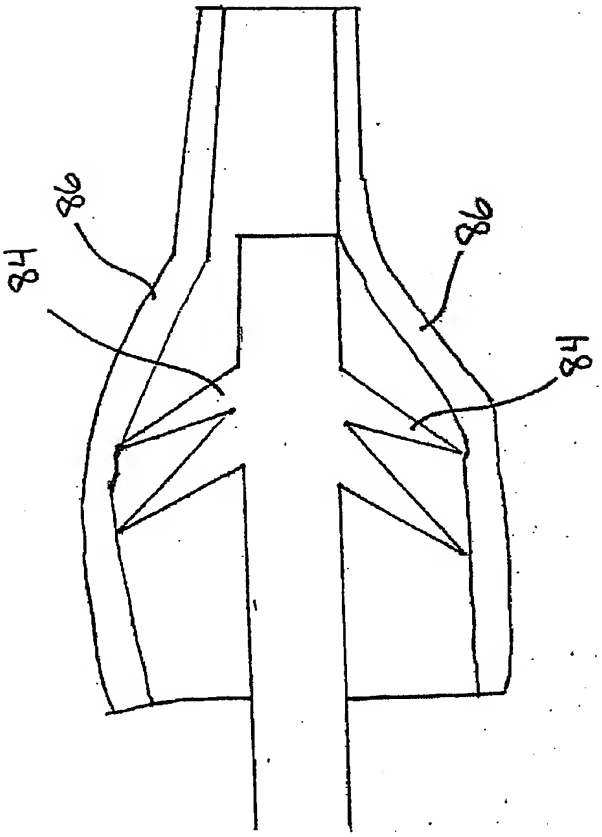
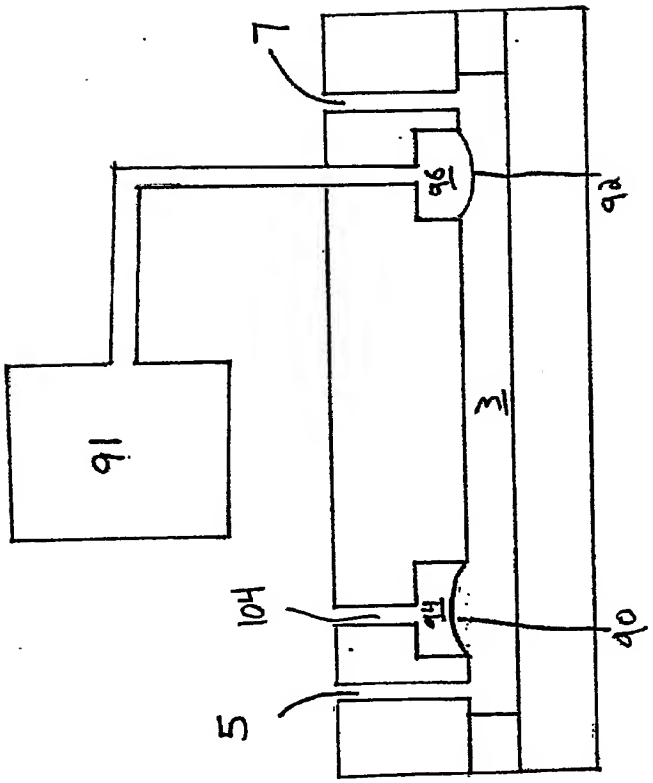


FIG. 9



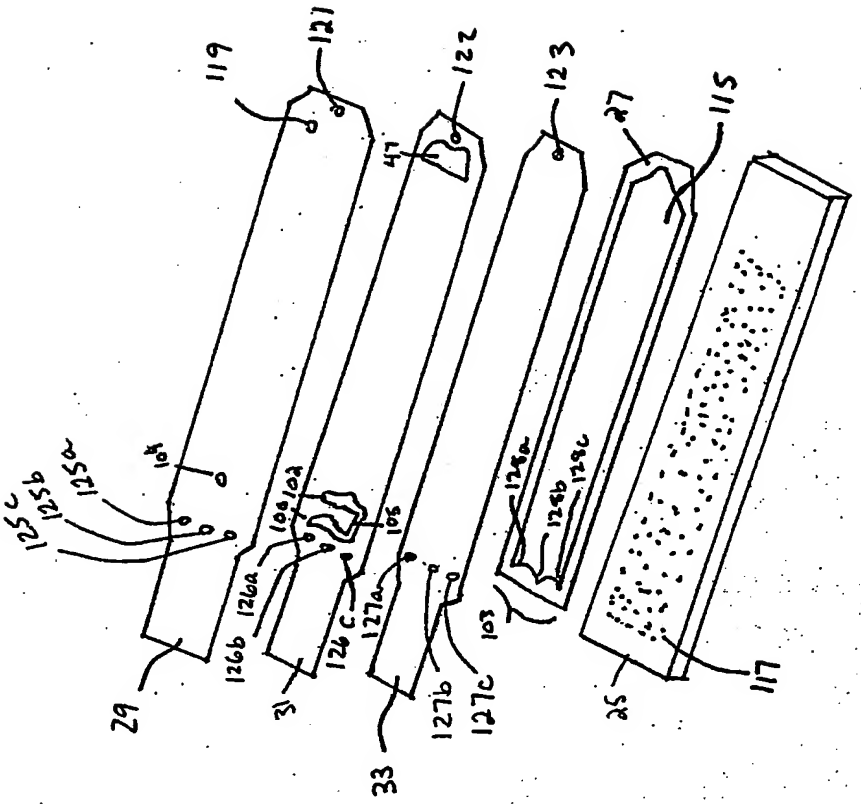
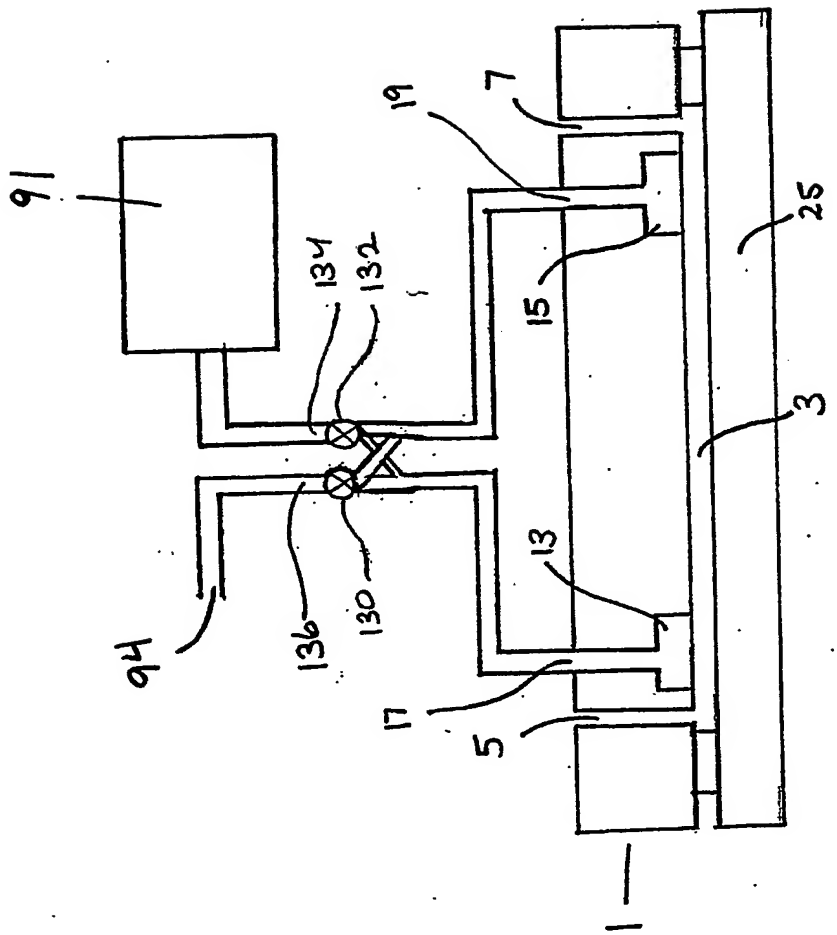


FIG. 10

FIG. 11



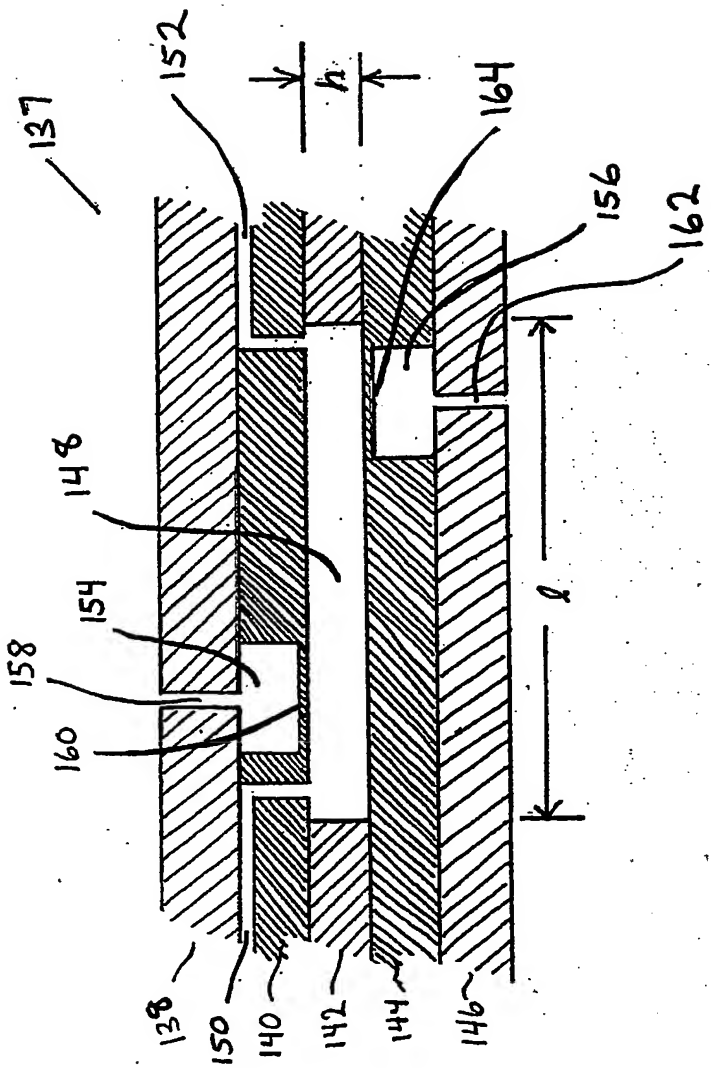
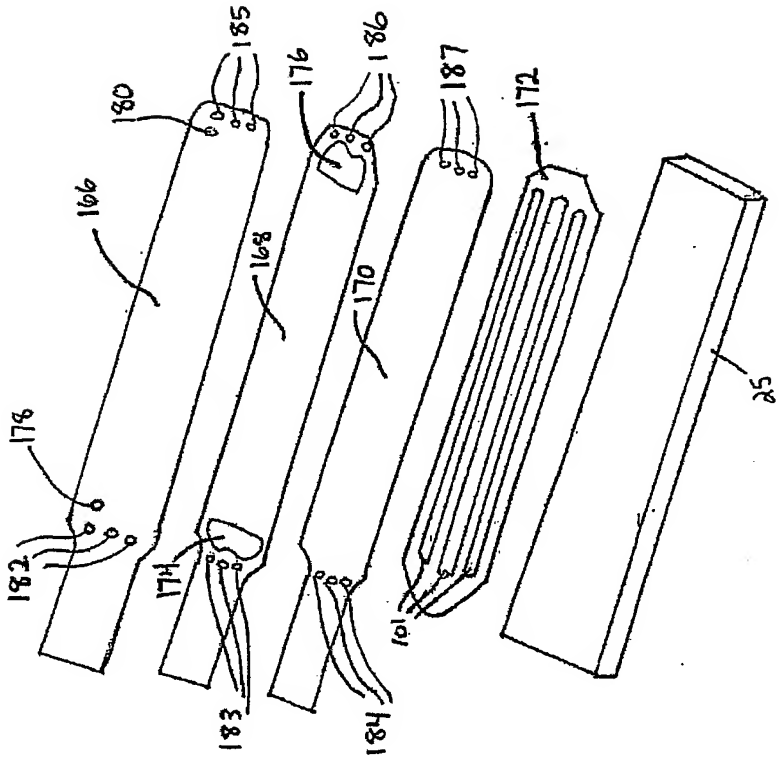


FIG. 12

FIG. 13



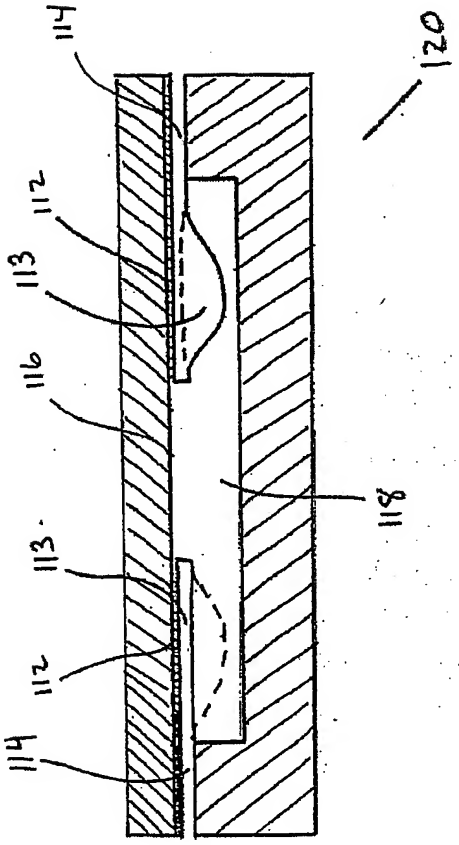


FIG. 14

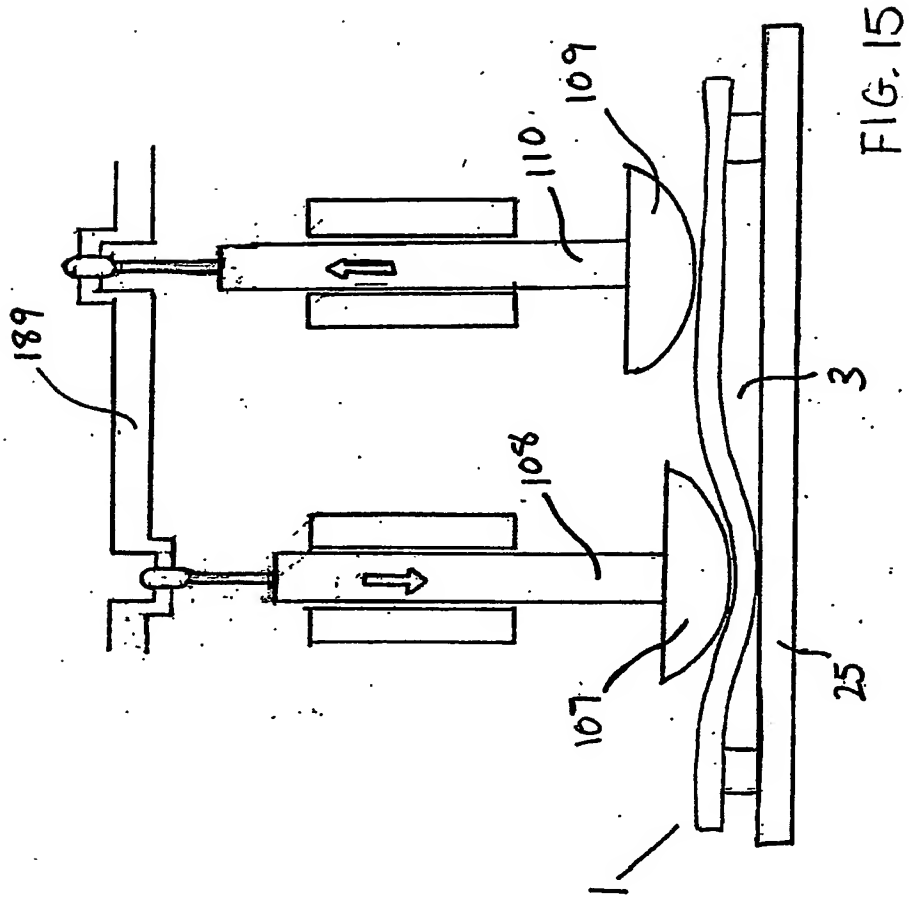


FIG. 15

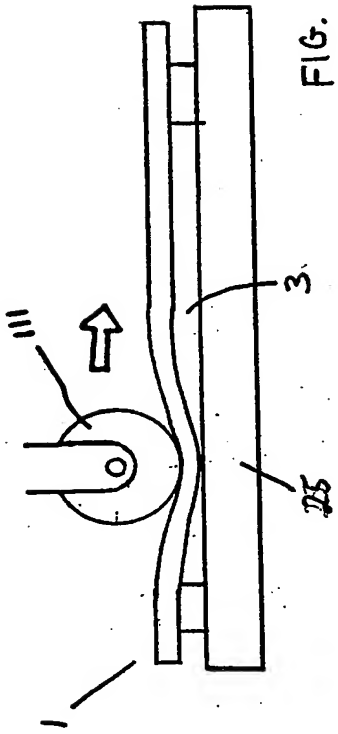


FIG. 16A

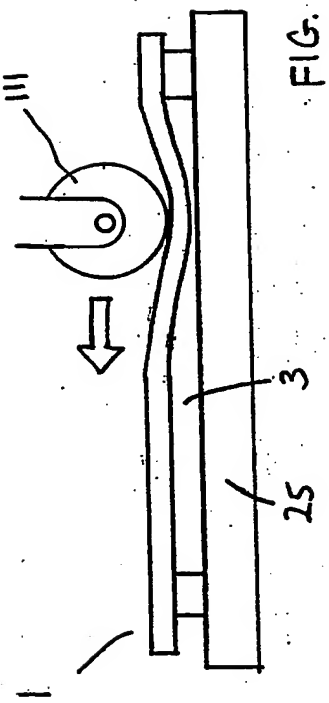


FIG. 16B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/25743

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : E01L 3/02, 3/00; F04B 43/10, 45/00, 43/06; B01F 13/02,11/00, 7/00, 13/00, 5/06

US CL : 422/100, 102, 99; 417/395, 394; 366/275, 280, 341, 336, 101, 114, 124

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/100, 102, 99; 417/395, 394; 366/275, 280, 341, 336, 101, 114, 124

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,902,096 A (BEHRINGER et al.) 11 May 1999, entire document	1 , 31, 51, 64, 82, and 88
A	US 5,100,626 A (LEVIN) 31 March 1992, entire document	1, 31, 51, 64, 82 and 88
A	US 4,687,423 A (MAGET et al.) 18 August 1987, entire document	1, 31, 51, 64, 82 and 88
A, P	US 6,399,394 B1 (DAHM et al.) 04 June 2002	1, 31, 51, 64, 82, and 88
A , P	US 6,376,256 B1 (DUNNINGTON et al.) 23 April 2002	1, 31, 51, 64, 82, and 88
A	US 5,718,567 A (RAPP et al.) 17 February 1998	1, 31, 51, 64, 82, and 88

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

05 November 2002 (05.11.2002)

Date of mailing of the international search report

03 DEC 2002

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

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Authorized officer

Jill Warden

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Form PCT/ISA/210 (second sheet) (July 1998)

PCT/US02/25743

INTERNATIONAL SEARCH REPORT

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,681,741 A (ATWOOD et al.) 28 October 1997	1, 31, 51, 64, 82, and 88
A	US 5,364,790 A (ATWOOD et al.) 15 November 1994	1, 31, 51, 82, and 88
A	US 5,346,672 A (STAPLETON et al.) 13 September 1994	1, 31, 51, 64, 82 and 88
A	US 4,494,912 A (PAULIUKONIS) 22 January 1985	1, 31, 51, 64, 82 and 88

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/25743

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-68

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/US02/25743

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-85 and 88 , drawn a mirco-fluidic device and methods of use.

Group II, claim(s) 86, drawn to a method of mixing fluid.

Group III, claim(s) 87, drawn to a method of mixing fluid.

The inventions listed as Groups I and (II, III) do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
The special technical feature for Group I is is a microfludic device that comprises two parallel walls, a perimeter wall, at least two flexible diaphragms, an inlet port, and mixing bladders, while the special technical feature for Group II is a method of mixing via a microfluidic device that comprises a chamber and a central wall, and for Group III is a method of mixing a fluid in a microfluidic device compring a chamber a wall, brayer, roller, or mechanical feet.
Groups II and III lack unity of invention because they do not require the specifics of the elements of Group I such as perimeter walls, main walls, diaphragms, mixing bladders, and etc.

Continuation of B. FIELDS SEARCHED Item 3:

East
Keyterms: diagraphm, bladder, gas, pressure, pressurized, gasket, main wall